

Nanoscale Biomimetic Modification of the Cell-Substrate Interface for Bone Tissue Engineering

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INTRODUCTION: Topographical modification may be particularly exploited in fracture fixation to improve osseointegration and implant stability. Cell-substrate interactions are mediated through transmembrane integrin receptors coupled to the extracellular matrix (ECM). Experimentally, nanofeatures have been shown to affect contact guidance *in vitro* and directly influence cellular adhesion¹. Here experimental nanotopographies of varying order have been fabricated by electron-beam lithography (EBL), photolithography (PL) and polymer demixing in polymethylmethacrylate (PMMA). This study is concerned with the effects these nanotopographies have on adhesion formation in S-phase osteoblasts.

METHODS: 100 nm deep nanopit topographies were fabricated by EBL in square (Sq), hexagonal (HEX) and near-square (N-Sq) symmetries. Grooved substrates produced by PL were 327 nm deep, 100 μm, 10 μm and 25 μm wide. Two Poly(styrene) and poly(bromostyrene) polymer demixed topographies were fabricated. Primary human osteoblasts (HOBs) were cultured on all substrates, and S-phase cells identified by bromodeoxyuridine (BrdU) labelling. Immunocytochemistry and SEM were used to observed vinculin, S-phase nuclei and F-actin. Adhesion size and number were quantified by Image analysis[#]. Adhesions were designated as focal contacts (FX), focal adhesions (FA) of fibrillar adhesions (FB) according to size².

RESULTS: Highly ordered arrays of nanopits disrupted cytoskeletal organisation and cellular adhesion relative to controls. Grooved substrates induced contact guidance and adhesion formation dependant on groove width. Both polymer demixed topographies induced cell flattening, but not increased adhesion formation, (Table 1.).

DISCUSSION & CONCLUSIONS: Polymer demixed substrates induced increased HOB spreading relative to controls; adhesion size and number however were not seen to increase. Adhesion formation on nanogrooves was increased with intergroove distance and cell density, possibly as a result of increased protein adsorption. Narrow grooves increased contact guidance and influenced adhesion orientation (Fig 1.). Nanotopographical

conformation can regulate adhesion formation and contact guidance.

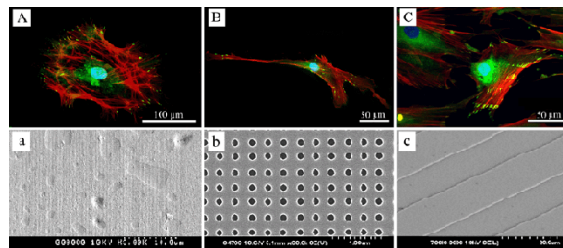


Fig. 1. (A) HOBs were spread on polymer demixed topographies (a). (B) Adhesion formation was reduced on Sq arrays of nanopits (b). (C) Grooved substrates influenced adhesion orientation (c).

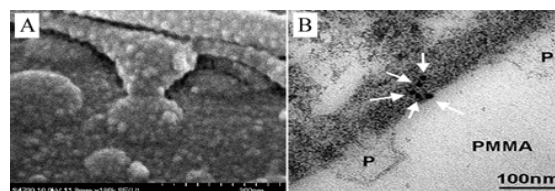


Fig. 2. (A) SEM of filopodial interaction with nanoislands. (B) Immuno-TEM interpit adhesion (P).

Table 1. Adhesion Subgroup Size and Number

Substrate Topography	Adhesion No			HOB Morphology
	FX	FA	FB	
Control	35	38	9	Spread
Square	25	15	2	Elongated
Hex	18	19	3	Rounded
N-Square	26	29	19	Spread
3% 1000 rpm	15	32	5	Spread
1% 3000 rpm	21	24	2	Spread
100 μm grooves	30	34	3	Spread
25 μm grooves	23	27	4	Elongated
10 μm grooves	14	25	3	Elongated

REFERENCES: [1] Zhu (2004) *Biomaterials* **25**, 4215-23. [2] Diener (2005) *Biomaterials* **26**, 383-392 [#]<http://rsb.info.nih.gov/nih-image>
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